Abstract for Origins of Tissue stem cells meeting

The involvement of EZH2 in regulation of osteogenic and adipogenic differentiation of human mesenchymal stem cells

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Background

Enhancer of Zeste homolog 2 (EZH2), a catalytic subunit of Polycomb repressive complex 2 (PRC2), transfer a methyl group from S-adenosyl methionine (SAM) to histone3 lysine27 (H3K27), resulting in gene silencing. It has been shown that EZH2 plays a pivotal role in regulating self-renewal and inhibiting the differentiation of embryonic stem cells. Also, EZH2 involve in the regulation of G2/M transition and cyclin-dependent kinase1 (CDK1) is one of the major G2/M kinase, and they are play an important role in controlling self-renewal and lineage determination of stem cells. Clinically, an increase in bone mass reduction has been observed in age-associated osteoporosis, which is accompanied by an accumulation of adipose tissue in bone marrow. Bone and fat are composed of osteoblasts and adipocytes, respectively, both of which are derived from multipotent bone marrow mesenchymal stem cells (MSCs). Furthermore, the differentiation of adipogenic and osteogenic lineages is mutually exclusive and requires sophisticate coordination between genetic and epigenetic processes. Disruption of the balance between MSC osteoblast and adipocyte differentiation is associated with various human diseases. Several transcription factors such as RUNX2 regulate osteogenesis. Overexpression of RUNX2 promotes differentiation of MSCs into osteoblasts. PPARγ-2, a member of the nuclear receptor superfamily, is required for adipogenesis. In addition, multipotent stem cells contain multiple key maternally inherited transcriptional factors that maintain the pluripotency, including epigenetic factors for histone modifications, such as the Polycomb group proteins EZH2**.**

Methods

Immunoprecipitation assay was performed to evaluate the interaction between EZH2 and CDK1. Moreover, the involvement of CDK1-EZH2 pathway in osteogenic differentiation of human MSCs was examined through knocking down CDK1 expression by CDK1 shRNAs. In addition, we used EZH2-ChIP-on-chip assay to identify differential EZH2 targets in the two differentiation stages on a genome-wide scale. After validating the targets, we found that HDAC9c/MITR was expressed in osteoblasts while greatly decreased in adipocytes.

Results

Activation of CDK1 is able to phosphorylate EZH2 at Thr 487. In human mesenchymal stem cells, phosphorylation of EZH2 at Thr 487 by activated CDK1 promoted MSC differentiated into osteoblasts. The phosphorylation of EZH2 at Thr 487 disrupted EZH2 binding with other PRC2 components SUZ12 and EED thus inhibited EZH2 methyltransferase activity to de-repress targeted genes such as RUNX2. Phosphorylation of EZH2 at Thr 487 resulting in fail targeted on RUNX2 promoter can enhance osteogenic differentiation of human MSCs. We also found that EZH2 bound to the HDAC9c/MITR promoter suppressing HDAC9c/MITR in adipocytes but not in osteoblasts. The presence of HDAC9c/MITR in osteoblasts promoted the formation of the MITR/HDAC9c formation of the HDAC9c/MITR and PPARγ-2 complex in the nucleus of osteoblasts inhibiting the transcriptional activity of PPARγ-2 and preventing adipocyte differentiation. Thus, HDAC9c/MITR functions as a transcriptional co-repressor in MSCs undergoing osteogenesis through interruption of PPARγ-2 transcriptional activity.

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